REMARKS

Summary of the Invention

The invention features methods for identifying the activity range of test compounds that modulate the biological activity of a CeSERT against non-CeSERT, secondary targets, and for identifying test compounds that modulate the uptake of serotonin by secondary targets other than a SERT.

Support for the Amendments

Support for the amendment to claims 1 and 12 is found on page 2, line 26, through page 3, line 4, of the specification. Claims 2-7 and 11 have been amended to place the claims in proper dependent format. Support for new claims 13-22 is found in prior claims 2-11. No new matter is added by the amendment.

Summary of the Office Action

Claims 1-12 are pending and stand rejected under 35 U.S.C. § 112, second paragraph, for lack of clarity. Claims 1-12 are also rejected under 35 U.S.C. § 112, first paragraph, for lack of written description and for lack of enablement. The Examiner also objects to the substitute declaration. By this reply Applicants amend claims 1 and 12, provide a new declaration, and address the Examiner's rejections and objection. Applicants respectfully request reconsideration of the claims.

Declaration

The Examiner states that the substitute declaration submitted by Applicants on May 23, 2002 is defective because the date of the second inventor's signature is incomplete. In reply, Applicants submit herewith a newly executed Declaration of the inventors. Accordingly, this objection should be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-12 are rejected under 35 U.S.C. § 112, second paragraph, for lack of clarity. The Examiner states that there is insufficient antecedant basis for the limitation "the defined behavior of a second nematode" in claims 1 and 12, and that claims 1 and 12 are unclear as written "because it is not clear if the defined behaviors of the first and second nematodes are the same." Office Action, p. 9. Applicants have amended claims 1 and 12 to clarify the claim language to clearly indicate that the method involves observing a single defined behavior by a treated nematode, relative to that same behavior by an untreated nematode, following contact of the treated nematode with a test compound. Accordingly, the rejection of claims 1-12 under 35 U.S.C. § 112, second paragraph, should be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Written Description

Claims 1-12 are rejected under 35 U.S.C. § 112, first paragraph, for lack of written description. The Examiner states that claims 1-12 contain "subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains,

or with which it is most nearly connected, to make and/or use the invention...The specification has disclosed *C. elegans* having a mutated mod-5 polypeptide comprising mod-5(n822), mod-5(n823), or mod-5(n3314) mutations...[, but] has not disclosed the other nematodes encompassed by the genus of nematode expressing a mutated CeSERT polypeptide." Applicants respectfully disagree.

The Presently Claimed Genus of Nematode is Fully Described

Applicants' invention is based on the identification of the sole *C. elegans* serotonin reuptake transporter (CeSERT), termed mod-5. Applicants have discovered that a nematode expressing a CeSERT polypeptide with a reduced ability to take up serotonin, relative to nematode expressing a normal CeSERT polypeptide, due to, e.g., one or more mutations or deletions in the CeSERT amino acid sequence, can be used in methods to identify compounds that stimulate a secondary target other than a CeSERT polypeptide, thereby promoting serotonin reuptake via another pathway. Applicants also recognized that the nematodes can be used in methods to identify side effects of compounds due to their modulation of a non-CeSERT, secondary target.

The Examiner asserts that although the present claims recite a genus of nematode that expresses a mutated CeSERT polypeptide with a reduced ability to sequester serotonin from the synapses of serotonergic neurons, the specification only provides an adequate written description of nematodes expressing mod-5(n822), mod-5(n823), and mod-5(n3314). The Federal Circuit, though, acknowledged that "every species in a genus need not be described in order that a genus meets the written description requirement." (*Regents of the University of California v. Eli Lilly*

and Co., 119 F.3d 1559, 43 U.S.P.Q.2d 1398, 1405 (Fed. Cir. 1997) (citing *Utter v. Hiraga*, 845 F.2d 993, 6 U.S.P.Q.2d 1709 (Fed. Cir. 1988)) ("A specification may, within the meaning of § 112, ¶ 1, contain a written description of a broadly claimed invention without describing all species that claim encompasses.")) The *Lilly* court further acknowledged that "it may not be necessary to enumerate a plurality of species if a genus is sufficiently identified ... by other appropriate language." *Lilly*, 119 F.3d at 1569. The M.P.E.P. § 2163(II)(A)(3)(ii) also sets forth this standard, stating:

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice..., reduction to drawing..., or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show applicant was in possession of the claimed genus... See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. (Citations omitted; emphasis added.)

Applicants have plainly met this standard.

The specification teaches that the genus of nematodes recited in the present claims are derived from a single species, *Caenorhabditis elegans*, and that they express a CeSERT polypeptide that is functionally deficient in serotonin reuptake activity, as determined using biochemical assays (see, e.g., page 15, line 22, through page 20, line 28, of the specification), and behavioral criteria (e.g., effects on locomotion, egg-laying, pharyngeal pumping, nose contraction, and defecation in response to deficits in serotonin reuptake. Applicants' specification also describes the identification and cloning of mod-5, the gene that encodes the CeSERT polypeptide. Applicants have disclosed the function of CeSERT as a serotonin

reuptake transporter and have placed it in the context of previously identified serotonin reuptake transporters. Furthermore, Applicants have confirmed that CeSERT is structurally similar to SERTs from other species (e.g., human and drosophila; see, e.g., Fig. 3B).

In the specification, Applicants also demonstrate that CeSERT has a number of highly conserved regions (see, e.g., Figure 3B). In addition, the specification states:

> MOD-5 protein is predicted to contain 12 putative transmembrane regions (Fig. 3*B*). Much of the sequence conservation is clustered in or around these transmembrane regions, suggesting that the membrane topology of the SERTs is important for their function. At position 119 of SEQ ID NO:5 (Fig. 3*B*, diamond), within the first predicted transmembrane domain, MOD-5 has an aspartate residue that is conserved in serotonin, dopamine, and norepinephrine (NE) reuptake transporters but not in gamma-aminobutyric acid (GABA) reuptake transporters...This aspartate may be involved in binding to the amino group in serotonin, dopamine, and NE... (Specification, page 19, lines 2-13.)

Therefore, Applicants recognized that the transmembrane topology of the CeSERT polypeptide is related to its ability to mediate serotonin binding and reuptake.

Applicants' confirmed the role of the transmembrane domain region of the CeSERT polypeptide by identifying three representative CeSERT polypeptide species, mod-5(n822), mod-5(n823), and mod-5(n3314), each of which contain a mutation in the transmembrane domain region (see, e.g., page 18, lines 27-31, and page 19, lines 26-29, of the specification). Accordingly, Applicants' specification not only identifies three representative species of the presently recited genus, it also provides a correlation between the functional property of the CeSERT polypeptide, i.e., serotonin reuptake, with a structural property of the polypeptides (i.e., the transmembrane domain). Furthermore, as is indicated in Figure 3B, there are several highly conserved structural regions within the transmembrane domain of the polypeptide. This

disclosure, in combination with the biochemical and behavioral assays described in the specification, provide relevant, identifying characteristics that one skilled in the art can use to identify species of nematode lacking serotonin reuptake activity due to a mutated CeSERT polypeptide that fall within the scope of the present claims.

The Examiner argues that because Applicants' specification describes only three nematodes with mutated CeSERT polypeptides, all others lack written description. Applicants disagree. The specification clearly establishes the metes and bounds of what is claimed. In particular, even though the claimed invention is exemplified by three nematodes expressing a mutated CeSERT polypeptide, one of skill in the art, upon reading the specification, would have readily recognized that these examples were provided for the purpose of illustrating the invention and that Applicants' invention included any nematode expressing a mutated CeSERT polypeptide that is structurally related and that exhibits reduced serotonin reuptake activity. The specification defines these related CeSERT polypeptides as, e.g., "any protein substantially identical to the CeSERT polypeptides of SEQ ID NOs: 5-8", other naturally-occurring *C. elegans* proteins, allelic variants, natural mutants, induced mutants, chimeric polypeptides, analogs that differ by amino acid sequence differences, post-translational modifications, or both, or truncated polypeptides (see, e.g., page 33, line 22, through page 34, line 23, of the specification). In view of these teachings, the specification clearly conveys Applicants' presently claimed invention to those persons of skill in the art. Furthermore, the specification clearly allows the skilled worker to identify and recognize other species of nematode that fall within the present claims.

Based on this description, one skilled in the art would recognize that Applicants' invention encompasses not only three nematodes expressing a mutated CeSERT polypeptide, but

rather, a family of nematodes that are related by defects in serotonin reuptake activity due to the presence of a mutation in their CeSERT polypeptide. Thus, there can be no question that Applicants were in possession of the claimed genus at the time their application was filed, and that one skilled in the art would recognize Applicants' disclosure as a description of the invention defined by claims 1 and 12, and their dependent claims. As a result, Applicants' specification clearly satisfies the written description requirement, as set forth by the case law and the M.P.E.P., and Applicants request reconsideration and withdrawal of the 35 U.S.C. § 112, first paragraph, rejection.

Enablement

Claims 1-12 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner states that claims 1-12 contain "subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention...The specification has disclosed *C. elegans* having a mutated mod-5 polypeptide comprising mod-5(n822), mod-5(n823), or mod-5(n3314) mutations...[, but] has not disclosed the other nematodes encompassed by the genus of nematode expressing a mutated CeSERT polypeptide." The Examiner also states that "the specification has failed to provide guidance that correlates a defined behavior with modulation of the uptake of serotonin by modulating the activity of a secondary target as embraced by the claims." Applicants respectfully disagree.

The Genus of Nematode with a Mutated CeSERT Polypeptide is Enabled

The Examiner asserts that the present claims lack enablement because "the specification fails to provide any relevant teachings or specific guidance with regard to the use of other nematodes expressing mutated CeSERT polypeptides embraced by the claims." Office Action, page 6. All that is required to satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph, is a description in the specification of how to make and use the invention (see, e.g., M.P.E.P. § 2164). Applicants have plainly met this standard.

The specification provides several methods for identifying nematodes expressing a mutated CeSERT polypeptide with reduced serotonin reuptake for use in the presently claimed methods. For example, the specification describes the formaldehyde-induced fluorescence (FIF) histochemistry assay in which nematodes are exposed to exogenous serotonin and assayed for FIF in the cell bodies of their neurosecretory motor neurons (NSMs; see, e.g., page 16, lines 10-22). Nematodes expressing a wild-type CeSERT polypeptide demonstrate FIF, while nematodes expressing a defective CeSERT polypeptide lack FIF due to an inability to take up serotonin. Therefore, the FIF assay can be used to easily identify nematodes lacking normal CeSERT function.

The specification also states that anti-serotonin antibodies can be used to identify nematodes expressing a defective CeSERT polypeptide (see, e.g., page 16, line 23, through page 17, line 3). Anti-serotonin antibodies are more sensitive than FIF in detecting serotonin in NSMs and do not require preincubation with exogenous serotonin. In fact, Applicants used both techniques to identify two of the three CeSERT mutants, mod-5(n822) and mod-5(n823), as is described in the specification (see page 16, lines 17-22, and page 17, lines 4-7).

The specification also teaches the use of behavioral assays for identifying nematodes expressing defective CeSERT polypeptides. For example, the assay described on page 21, lines 1-28, can be used to identify nematodes expressing a mutated CeSERT polypeptide based on the serotonin-dependent enhanced slowing response of nematodes in the presence of a food source. The specification teaches that "whereas well-fed wild-type animals [i.e., nematodes] slow their locomotory rate slightly in response to bacteria (the basal slowing response), food-deprived wild-type animals display a greater degree of slowing their locomotory rate in response to bacteria (the enhanced slowing response)." (See page 21, lines 10-13.) The specification further teaches that food-deprived nematodes defective in serotonin uptake exhibit a hyperenhanced slowing response relative to food-deprived wild-type nematodes. Therefore, nematodes defective in CeSERT function can be easily identified by the observation of a hyperenhanced slowing response in their locomotory rate in the presence of food.

Given the biochemical and behavioral assays provided in the specification, as is discussed above, one skilled in the art could easily identify additional nematodes expressing a defective CeSERT polypeptide for use in the presently claimed methods. Further confirmation that the nematodes identified using the methods provided in the specification express a mutated CeSERT polypeptide can be obtained by comparing the mod-5 gene or polypeptide sequence of the putative mutant nematode with the wild-type mod-5 gene or polypeptide sequence described in Applicants' specification (see, e.g., SEQ ID NO: 1 or SEQ ID NO: 5). Therefore, Applicants' specification clearly describes how to "make" the invention.

Applicants' specification also teaches how to "use" the invention. The specification teaches methods, discussed above, which can be used to identify the activity range of a test

compound against a secondary target (i.e., a non-CeSERT target) by assaying various concentrations of the test compound using a nematode expressing a defective CeSERT polypeptide and observing the behavior of the nematode, relative to a nematode not contacted with the test compound (see, e.g., page 37, line 1, through page 38, line 25, and Examples 3 and 5). Because the mutant nematode is defective in serotonin reuptake, the methods described in the specification provide a convenient system for identifying potential side effects of a test compound, e.g., an existing serotonin selective reuptake inhibitor or other therapeutic compound, based on its interaction with a non-CeSERT, secondary target.

As an example, the specification describes contacting wild-type nematodes and nematodes expressing a defective CeSERT polypeptide with fluoxetine, which is a CeSERT inhibitor. In the presence of varying concentrations of fluoxetine, both the wild-type and CeSERT-defective nematodes displayed several defined behaviors, including, e.g., paralysis, nose muscle contraction, and egg-laying (see, e.g., page 30, line 25, through page 35, line 20). The presence of these behaviors in both groups of nematodes, upon contact of the nematodes with fluoxetine, indicates that fluoxetine acts on another target in addition to CeSERT (i.e., a secondary target). Therefore, using the methods of the invention, the activity range of a test compound, i.e., the concentration at which the test compound elicits a defined behavior in the nematode, can be determined by increasing or decreasing the concentration of the test compound based on the response of the nematode contacted with the test compound.

The specification also describes the use of nematodes expressing a defective CeSERT polypeptide for identifying a test compound that modulates serotonin reuptake by acting on a secondary target that is not a CeSERT. For example, the specification describes contacting a

nematode expressing a mutated CeSERT polypeptide, which has a reduced capacity to take up serotonin relative to a wild-type nematode, with a test compound, followed by observing a defined behavior of the nematode (e.g., liquid locomotion, pharyngeal pumping, egg-laying, nose contraction, and defecation behaviors; see, e.g., page 2, line 26, through page 3, line 4, and Examples 1, 2, 4, and 5). A change in the behavior of the nematode, relative to a second nematode expressing a mutated CeSERT polypeptide, but not contacted with the test compound, indicates that the compound modulates serotonin uptake by acting on a secondary target. The method identifies useful test compounds that can circumvent the serotonin reuptake defect caused by a mutated CeSERT polypeptide by acting on a non-CeSERT, secondary target to promote serotonin reuptake. The method simply requires assaying, by observation, treated and untreated nematodes expressing a mutated CeSERT polypeptide, and noting a change in a single behavior of the treated nematode, relative to the untreated nematode.

Accordingly, nematodes expressing a mutated CeSERT polypeptide completely lacking the ability to transport sertonin (e.g., mod-5 (n3314)) are well suited for use in the methods of the present invention because these nematodes can be used to assay test compounds for an effect on a secondary target without interference from any residual serotonin transport activity due to the mutated CeSERT polypeptide. Therefore, contrary to the Examiner's statement on page 8 of the present Office Action, claims 1-12 are enabled for the use of mod-5(n3314) even though a nematode expressing this mutated CeSERT polypeptide fails to demonstrate serotonin transport (see, e.g., pages 25-27 of the specification).

The Specification Clearly Describes a Correlation Between a Defined Behavior and the Effects of a Test Compound on a Nematode Expressing a Mutant CeSERT Polypeptide

The Examiner also asserts that "the specification has failed to provide guidance that correlates a defined behavior with modulation of the uptake of serotonin by modulating the activity of a secondary target as embraced by the claims" (Office Action, page 6). Contrary to the Examiner's statement, the specification teaches several defined behaviors which can be examined in nematodes expressing a defective CeSERT polypeptide, and which can be altered by modulating the activity of a non-CeSERT, secondary target.

Applicants point out that the presently claimed methods involve contacting a nematode expressing a mutated CeSERT polypeptide with a test compound and comparing the resulting behavior of the contacted nematode to the behavior of a nematode not contacted with the test compound. The behavior can result from the inability of the CeSERT-defective nematode to effectively clear serotonin from the synapses of serotoneurgic neurons, in which case contact with the test compound results in a loss of that behavior by modulating a non-CeSERT, secondary target that promotes serotonin clearance. Alternatively, the behavior can occur as a side effect resulting from contact of the nematode with a test compound due to the modulation of a non-CeSERT, secondary target.

The specification describes several behaviors that one skilled in the art can monitor. For example, the specification teaches that food-deprived nematodes expressing a wild-type CeSERT slow their locomotory rate slightly in response to a food source (e.g., bacteria), whereas food-deprived nematodes defective in serotonin reuptake due to a mutated CeSERT polypeptide exhibit a hyperenhanced slowing response when exposed to bacteria (see, e.g., page 21, lines 7-

28). The specification states that this behavior is a consequence of a defect in the clearing of serotonin from the relevant synapses (see page 21, lines 24-28). Therefore, the loss of the hyperenhanced slowing response as a result of contacting a nematode with a defective CeSERT polypeptide with a test compound indicates that the test compound modulates the activity of a non-CeSERT, secondary target, thereby circumventing the serotonin reuptake defect associated with the mutated CeSERT polypeptide.

The specification also teaches that exogenous serotonin inhibits locomotion in nematodes expressing a wild-type CeSERT polypeptide, and further that nematodes defective in serotonin reuptake due to the expression of a mutated CeSERT polypeptide are hypersensitive to exogenously added serotonin (i.e., paralyzed; see page 22, line 29, through page 23, line 3). The specification states that this response is due to the inefficient clearance of serotonin from the relevant synapses (see page 23, line 3). Accordingly, the loss of serotonin-mediated paralysis in a CeSERT-defective nematode contacted with a test compound indicates that the test compound modulates a non-CeSERT, secondary target.

Finally, the specification states that nematodes expressing a mutated CeSERT polypeptide are hypersensitive to exogenous serotonin in an egg-laying assay, and thus, these nematodes lay more eggs due to their inability to effectively clear serotonin from their synapses (see, e.g., page 32, lines 18-24, and Figs. 7A and 7B). Therefore, a decrease in egg-laying by a CeSERT-defective nematode contacted with a test compound indicates that the test compound modulates a non-CeSERT, secondary target, thereby overcoming the defect in serotonin clearance.

In sum, Applicants submit that the specification clearly describes and enables the full

scope of the present claims. The specification describes three exemplary CeSERT-defective nematodes, mod-5(n822), mod-5(n823), and mod-5(n3314), for use in the methods of the present claims, and provides several biochemical and behavioral assays for identifying additional CeSERT-defective nematodes. Finally, the specification clearly correlates a change in several defined behaviors of nematodes that occurs as a result of defects in serotonin uptake with modulation of a non-CeSERT, secondary target, and provides considerable guidance for one skilled in the art to monitor these change using the methods of the invention.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

Enclosed is a Petition to extend the period for replying for three months, to and including October 9, 2003, and a check for the fee required under 37 C.F.R. § 1.17(a). Also enclosed is a check for the fee required under 37 C.F.R. § 1.16(j) for the presentation of two claims in excess of twenty.

If there are any other charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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